

Development of the electrochemical biosensor for organophosphate chemicals using CNT/ionic liquid bucky gel electrode

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A B S T R A C T

Organophosphorus hydrolase (OPH) immobilized on CNT/ionic liquid (IL) electrodes were prepared by using three different intrinsic kinds of ILs, as binders. CNTs/ILs lead to dramatic electrochemical enhancements with respect to response time, stability, and sensitivity of composite electrodes. In addition, the electrochemical and biocatalytic properties of three-composite electrodes were strongly influenced by different types of ILs used, as verified by cyclic voltammetry and chronoamperometry. These results were attributed to the conformational changes of the microenvironment between the OPH and the composite electrodes within three different types of ILs. In particular, the biocatalytic signals of three OPH/CNT/ILs-modified electrodes increased linearly to the concentration of paraoxon in a wide range of 2–20 μ M. These findings provide a deep understanding of the role of each IL on the modified electrodes, enabling to enhance electrochemical properties for biosensors.

Keywords:

Biosensor

Organophosphorus hydrolase

Ionic liquid

CNT

Electrochemical property

1. Introduction

Detection of organophosphate (OP) compounds has attracted much attention in terms of safeguarding human health owing to their frequent use as pesticides in agriculture and their potential use as chemical warfare agents [1]. Among a variety of biological methods based on the biocatalytic activity of organophosphorus hydrolase (OPH), amperometric, potentiometric, and optical biosensing devices have been developed for detecting OPs [2–4]. Electrochemical biosensors in particular have been widely investigated to monitor various pesticides including OP compounds such as paraoxon [1], parathion [5], sarin [6], and soman [6] via an enzyme-catalyzed hydrolysis reaction by OPH due to their fast speed, high efficiency, low cost, and small sample size [1]. Chen et al. developed OPH amperometric biosensors with carbon electrodes for monitoring OP pesticides [5]. Wang et al. reported on OPH/carbon nanotube (CNT) amperometric biosensors wherein the electrodes were modified with CNTs for enhancing the performance of enzyme activity [7]. The widespread interest in OPH-based electrochemical biosensors stems from their simplicity, directness, and speed for determining OPs.

Considering the requirements for the detection of OPs, CNTs have been considered as potential electrode materials of electro-

chemical biosensors due to their high accessible surface area, electronic conductivity, stability, and capacity to immobilize enzymes [7]. Bucky gels (BGs) consisting of ionic liquids (ILs) and CNTs have served as advanced electrode materials of electrochemical biosensor devices since they were first reported by the Aida group [8]. They offer good biocompatibility, ease of preparation, chemical and environmental stability, and high dispersion of CNTs in ILs [8,9]. Dong et al. demonstrated efficient electron transfer between a CNT/IL-modified electrode and a protein [9]. Yao et al. reported the direct electrochemistry of heme proteins by using glass carbon electrodes modified with the gels of CNTs and water-miscible ILs [10]. However, there have been very few reports regarding the underlying relationship of the electrochemical properties of CNT/IL-composite electrodes according to the types of ILs for developing biosensors. Furthermore, no work has been reported on the application of CNT/IL electrodes to detect OP compounds.

Herein, we report on multi-walled CNT (MWNT)/IL-modified gold (Au) electrodes fabricated using three kinds of ILs, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF₄]), 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF₆]), and 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([bmim][Tf₂N]), as the binder. OPH/MWNT/IL/Au electrodes were fabricated by directly immobilizing OPH on MWNT/IL-modified Au electrodes, and their electrochemical and electrocatalytic behaviors were subsequently investigated by cyclic voltammetry (CV) and chronoamperometry.

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Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01 APR 2010		2. REPORT TYPE Final		3. DATES COVERED 23-06-2008 to 23-09-2009	
4. TITLE AND SUBTITLE Development of ubiquitous carbon-nanotube-based sensor for detection of organophosphate residues				5a. CONTRACT NUMBER FA23860814009	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Sang Yup Lee				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Korea Advanced Institute of Science and Technology,373-1 Guseong-dong, Yuseong-gu,Daejeon,Korea (South),KR,305-701				8. PERFORMING ORGANIZATION REPORT NUMBER N/A	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Asian Office of Aerospace Research & Development, (AOARD), Unit 45002, APO, AP, 96338-5002				10. SPONSOR/MONITOR'S ACRONYM(S) AOARD	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) AOARD-084009	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
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15. SUBJECT TERMS chemical sensors, Carbon nano tubes					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 4	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

2. Experimental section

2.1. Materials

High-purity MWNTs (HiPco, >95%) were obtained from Carbon Nanotechnologies. 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, and 1-butyl-3-methylimidazolium hexafluoro phosphate were supplied with high purity of 99.9% by C-TRI. Other chemicals were purchased from Sigma–Aldrich.

OPH was used as a model protein for the immobilization onto the CNT/IL gel electrode and the hydrolysis of the OP substrate. For the cloning of mature OPH (mOPH) gene, PCR amplification was carried out using the primers P1 (5'-GAAATTCATATGCATCATCACCACCACCGATCGATCGGCACAGGCGA-3') and P2 (5'-AAACTCGAGTGACGCCGCAAGGTGGTGA-3'), and the genomic DNA of *Flavobacterium* sp. ATCC27551 as a template. The PCR product of the mOPH gene was digested with *NdeI* and *XhoI*, and then ligated into the same sites of pET-22b(+) to construct pET-mOPH.

For the production of mOPH, *Escherichia coli* BL21(DE3) (*F[−]ompT hsdS_B (r_B[−] m_B[−]) gal dcm* (DE3)) harboring pET-mOPH was cultivated in 100 mL Luria-Bertani medium supplemented with ampicillin (100 µg mL^{−1}) in a shaker (37 °C, 200 rpm). After the addition of isopropyl-β-D-thiogalactopyranoside for protein expression, cells were further cultivated for 6 h and disrupted by sonication (Braun Ultrasonics) for 1 min at 20% output. After centrifugation (16,000g, 10 min, 4 °C), the insoluble pellet fraction was saved for the protein purification.

2.2. Preparation of MWNT/ILs-modified Au electrodes

Three types of BG-modified Au electrodes, MWNT/[bmim][BF₄]/Au electrode (MBGE), MWNT/[bmim][PF₆]/Au electrode (MPGE), and MWNT/[bmim][Tf₂N]/Au electrode (MTGE), were prepared as follows. Prior to use, the Au electrodes were pretreated by first polishing with 0.05 µm alumina powder and then ultrasonically cleaned with deionized water. All of the pretreated Au electrodes were dried at room temperature under vacuums. To obtain MWNT/IL gels, 5 mg of MWNTs were suspended in 0.1 mL of [bmim][BF₄], and the mixture grounded with agate mortar for 20 min. MWNT/[bmim][PF₆] and MWNT/[bmim][Tf₂N] gels were prepared by this same method. The MWNT/ILs/Au electrodes were prepared by mechanically rubbing MWNT/IL gels on the Au elec-

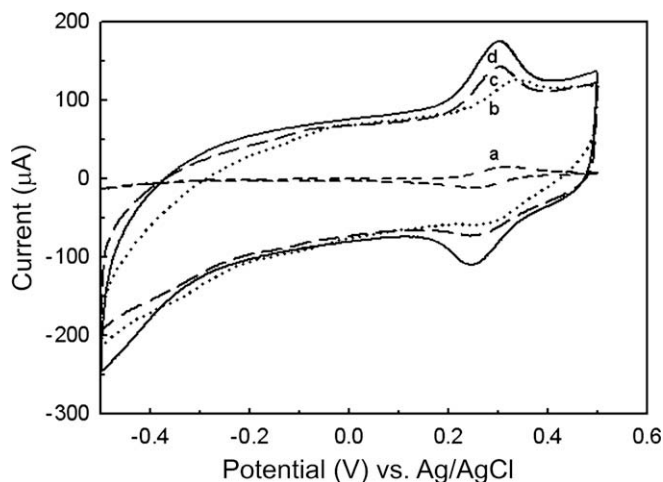


Fig. 1. Cyclic voltammograms in solution containing 5 mM K₃Fe(CN)₆ in 0.1 M KCl at a scan rate of 100 mV s^{−1}. (a) Bare gold electrode, (b) MBGE, (c) MPGE, and (d) MTGE.

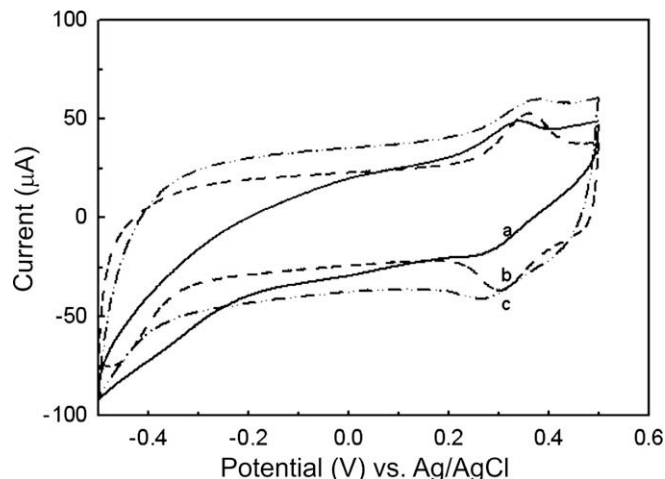


Fig. 2. Cyclic voltammograms in solution containing 5 mM K₃Fe(CN)₆ in 0.1 M KCl at a scan rate of 100 mV s^{−1}. (a) OPH/MBGE, (b) OPH/MPGE, and (c) OPH/MTGE.

trode surface. All of the modified Au electrodes were coated with 0.5 mg of MWNT/[bmim][BF₄], MWNT/[bmim][PF₆], and MWNT/[bmim][Tf₂N] to investigate the influence of ILs, and excluded the influence of their content. To immobilize the enzyme on the surface of the modified electrodes, the MWNT/IL-modified Au electrodes were immersed into phosphate-buffered saline (PBS, pH 7.4) containing 5 mg mL^{−1} of OPH for 12 h and then rinsed with PBS to remove loosely bound enzyme molecules and MWCNTs.

2.3. Instrumental methods

CV scans were recorded using a CHI660C electrochemical workstation (CH Instruments) with a conventional three-electrode electrochemical cell using an Au working electrode, a KCl–Ag/AgCl reference electrode, and a platinum wire counter electrode. The electrochemical measurements were performed in range from −0.5 to +0.5 V to investigate the typical cyclic voltammograms of electrodes and the baseline of the background current. All potentials were collected versus a KCl–Ag/AgCl reference electrode. The chronoamperometric responses were performed in N₂-saturated PBS at constant potential. All electrochemical measurements were performed at room temperature.

3. Results and discussion

3.1. Electrochemical properties of MWNT/IL/Au electrodes

Fig. 1 depicts the cyclic voltammograms of the bare and modified Au electrodes. Higher current responses of the modified Au electrodes compared to those of the Au electrode are attributed to the accelerated electron transfer and the large available surface area of the highly dispersed MWNTs in BGs [9]. The oxidation potential (E_{pa}), reduction potential (E_{pc}), peak-to-peak potential separation (ΔE_p), and formal potential [$E^0 = (E_{pa} + E_{pc})$] were also influenced by the type of MWNT/IL. When the bare Au electrode was modified with the three types of BGs, ΔE_p dramatically decreased from 69 mV for the bare Au electrode to 65 mV for the MBGE, 58 mV for the MPGE, and 50 mV for the MTGE. These findings elucidate that the electrochemical reactions of the MPGE and MTGE are more reversible than that of the MBGE. Given that the electron transfer-promoting effect of ILs depends on the structure of anions [11] and the interface interactions between the Au electrode surface and the different types of BGs [12], [bmim][PF₆] and [bmim][Tf₂N] are more efficient binders than [bmim][BF₄] for the

fabrication of the modified electrode. Stability of three-composite electrodes was investigated through an operation of 100 cycles in a potential ranging from -0.5 to $+0.5$ V. Absence of any significant change of the current response on composite electrodes indicates that they have excellent electrochemical stability. Furthermore, the three modified electrodes retained 90% of the initial current after immersion for 2 weeks in PBS at 4°C . In order to gain further insight into the relationship between the electrochemical properties and the types of ILs, cyclic voltammograms of OPH/MWNT/IL/Au electrodes were analyzed after immobilization of OPH on three-composite electrodes (Fig. 2). A decrease in the peak currents of the composites was observed, indicating that the electrochemical reaction at the electrode surface was inhibited by the enhanced charge transfer resistance through the immobilization of OPH. The

values of ΔE_p related to the electron transfer kinetics were influenced by ILs in the following order: OPH/MPGE (56 mV) < OPH/MBGE (63 mV) < OPH/MTGE (72 mV). The peak currents increased according to the following sequence: OPH/MTGE < OPH/MBGE < OPH/MPGE. The OPH/MPGE showed the lowest ΔE_p , indicating that it has a higher electron transfer rate compared to other modified electrodes, which is likely attributed to the favorable conformational effects after immobilizing OPH such as, biocompatibility, interfacial property, and macroscopic structure, as well as the effect of the counter anions of imidazolium ILs on the electron transfer reaction [11]. $E^{o'}$ values of three OPH/MWNT/IL/Au-composite electrodes also increased relative to those of the MWNT/IL-modified electrodes. Considering that the $E^{o'}$ is closely related

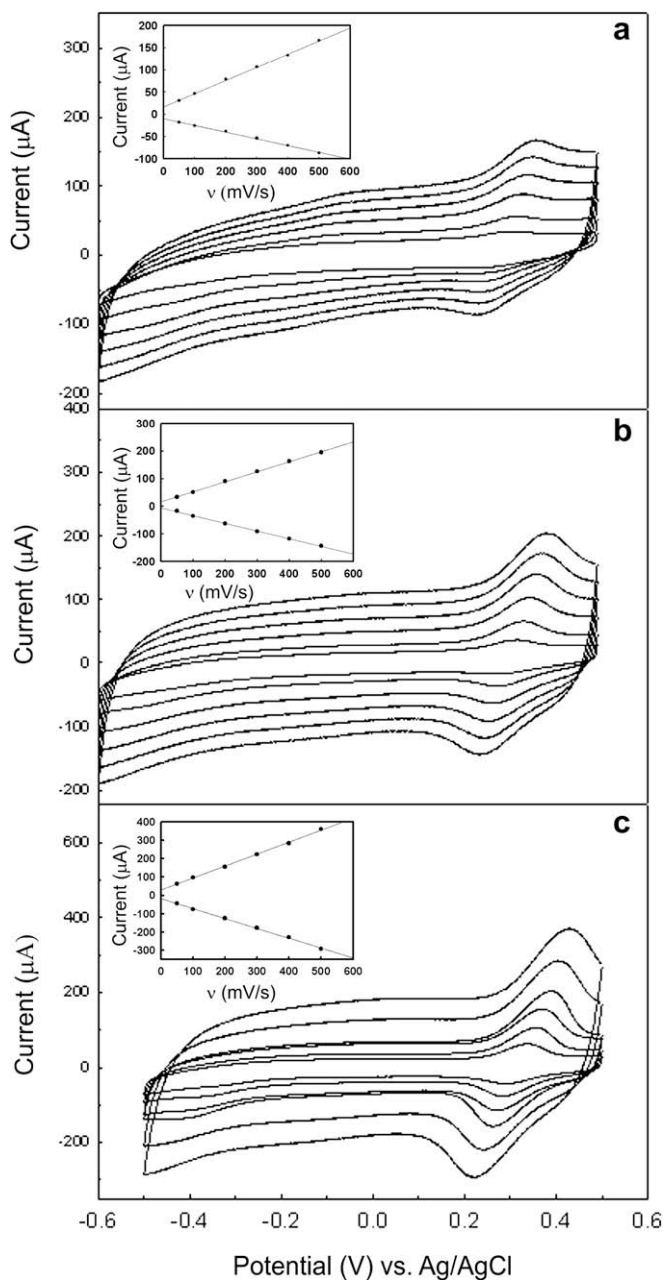


Fig. 3. Cyclic voltammograms of OPH/MWNT/IL/Au electrodes in solution containing N_2 -saturated PBS at different scan rate ($50, 100, 200, 300, 400$ and 500 mV s^{-1}). (a) OPH/MBGE (Inset is linear relationship of I_p and v at OPH/MBGE), (b) OPH/MPGE (Inset is linear relationship of I_p and v at OPH/MPGE), and (c) OPH/MTGE (Inset is linear relationship of I_p and v at OPH/MTGE).

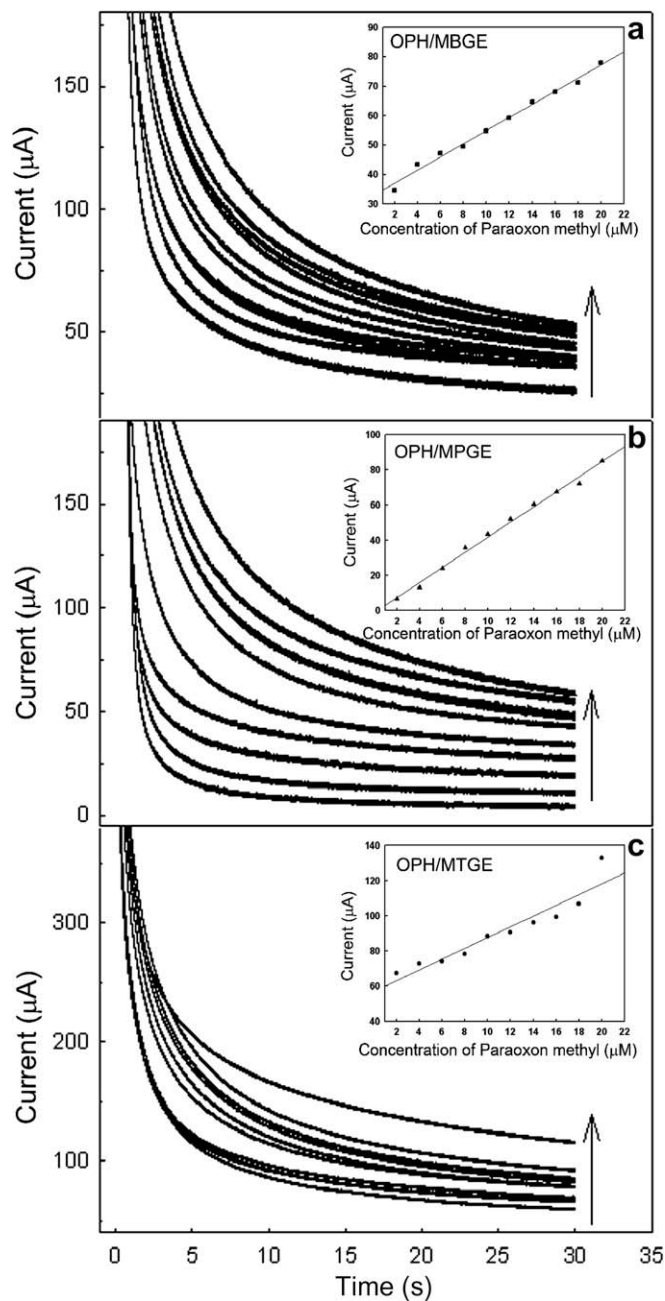


Fig. 4. Chronoamperometric response with respect to $2\text{ }\mu\text{M}$ increments of paraoxon in N_2 -saturated PBS. Applied potential: $+0.85\text{ V}$. (a) OPH/MBGE, (b) OPH/MPGE, and (c) OPH/MTGE. The inset shows the calibration curves of the electrocatalytic current on the concentration of paraoxon at (a) OPH/MBGE, (b) OPH/MPGE, and (c) OPH/MTGE.

to a pair of redox peaks, it can be concluded that electrochemical equilibrium at the interface of the electrolyte and electrode was changed after immobilization of OPH onto the electrode surface.

Fig. 3 presents the cyclic voltammograms of OPH/MWNT/IL/Au electrodes at a series of scan rates ranging from 50 to 500 mV s⁻¹. The anodic and cathode currents of three-composite electrodes were linearly proportional to the scan rate, indicating that the electrochemical reaction is dominated by a surface controlled quasi-reversible process [13]. As the scan rate was increased, the anodic and cathodic currents of three electrodes were enhanced in the following increasing order: OPH/MBGE < OPH/MPGE < OPH/MTGE.

3.2. Electrocatalytic activity of OPH/MWNT/IL/Au electrodes

The electrocatalytic activity of OPH immobilized on three-composite electrodes was investigated by detecting the hydrolysis product of paraoxon in a range of 2–20 µM. Fig. 4 shows the chronoamperometric responses of the OPH/MWNT/IL/Au electrodes with respect to the increments of 2 µM intervals in N₂-saturated PBS. Each chronoamperometry measurement was performed at +0.85 V as an optimum potential for biosensor operation by hydrodynamic voltammetric study. The OPH/MWNT/IL-modified electrodes showed high current and fast response time (~10 s) to reach a steady-state current. Sensitivities of three electrodes for the detection of paraoxon were derived from a linear calibration of concentration versus current, as shown in the insets of Fig. 4. The respective sensitivities correspond to 4.37 µA µM⁻¹ for OPH/MPGE, 2.46 µA µM⁻¹ for OPH/MTGE, and 2.40 µA µM⁻¹ for OPH/MBGE. These sensitivity values of the OPH/MWNT/IL-modified electrodes are much higher than those of OPH immobilized on a Nafion–carbon electrode (1.67 nA µM⁻¹) [5] and a CNT–carbon composite electrode (25 nA µM⁻¹) [7], respectively.

4. Conclusion

We herein described the development of MWNT/IL-modified electrodes for the electrochemical detection of OP. Modification

of MWNTs by ILs resulted in high dispersion of CNT bundles and the formation of a 3D-network structure, good compatibility with OPH, and accelerated electron transfer reaction at the interface. Electrochemical properties and sensor performances of composite electrodes depend on the types of ILs. In particular, electron transfer rate, indicated by ΔE_p , of the three modified electrodes increased after the immobilization of OPH on the modified electrode surface in the following order: OPH/MPGE < OPH/MBGE < OPH/MTGE. Among three-composite electrodes, MPGE provided the best sensitivity, 4.37 µA µM⁻¹, and a fast response time of ~10 s for detecting OP. Our composite electrodes could be applied to various electrochemical biosensors, based on their fast, stable, and sensitive properties.

Acknowledgments

This work was supported in part by the IT Leading R&D Support Project from the MKE through IITA, and the Korean-AFOSR Nanoscience and Technology Initiative from the US Air Force.

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